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13. ABSTRACT (Maximum 200 words)

Case reports of benzocaine-induced methemoglobinemia following application of topical anesthetics have been noted for man, cats, dogs, and sheep. Elevated levels of methemoglobin (MHB) could be a possible confounding variable for several types of experimental studies. We designed a screening study using a common topical benzocaine-containing anesthetics. Response to benzocaine spray occurred in most individuals tested, with response peaking between between 15 and 30 min after dosing. A positive MHB response ranged from 3.5% to 38% and occurred in over 95% of individual animals and occurred from 15-60 min after drug administration. Responses were quite variable due to the screening nature of the study and the topical route of drug administration but the highest responses occurred in rabbits and cats, the lowest in mice and dogs. MHB could be a confounding variable for several types of studies; investigators should consider this toxicity of benzocaine-containing topical anesthetics and use appropriate alternative methods or drugs (i.e. lidocaine).

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Benzocaine-induced methemoglobinemia attributed to topical application of the anesthetic in several laboratory animal species

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Summary

In a screening study, a common benzocaine-containing anesthetic was topically applied to the following species: dogs (n = 11), domestic shorthair cats (n = 38), Long-Evans rats (n = 22), Sprague-Dawley rats (n = 11), ferrets (n = 6), rhesus monkeys (n = 10), cynomolgus monkeys (n = 10), owl monkeys (n = 10), New Zealand White rabbits (n = 18), miniature pigs (n = 9), ICR mice (n = 4), C3H mice (n = 4), and C57BL/10SnJ mice (n = 24). All animals, except mice and rats, received a 2-second spray to the mucous membranes of the nasopharynx for an estimated dose of 56 mg. A 2-second spray to rodents' oral mucous membranes delivered too great a volume of fluid for these animals; therefore, an equivalent dose was applied to the oral mucosa membranes by use of a 23-gauge needle and syringe. Initial (baseline) blood samples, as well as 4 blood samples taken every 15 minutes after drug application, were analyzed for methemoglobin (MHb), using an oximeter. Positive MHb response (> 3 SD above baseline) was seen in individuals of all groups. The study was repeated in dogs several months later to confirm low response. Response to benzocaine spray was observed in most animals tested, with response peaking between 15 and 30 minutes after dosing. Positive MHb response ranged from 3.5 to 38%, was detected in > 95% of individual animals, and ranged from 15 to 60 minutes after drug administration. Responses were variable because of the screening nature of the study and the topical route of drug administration,

but the highest responses were observed in rabbits and cats, and the lowest were seen in mice and dogs. Methemoglobin could be a confounding variable for several types of studies; investigators should consider this toxicity of benzocaine-containing topical anesthetics and use appropriate alternative methods or drugs (ie, lidocaine).

Cats,^{1,2} dogs,³ and people^{4,5} may develop clinical methemoglobinemia after topical application of benzocaine-based drugs. Methemoglobin (MHb), the oxidized form of heme iron (Fe³⁺), does not carry oxygen (O₂), but increases affinity for O₂ by the remaining ferrous (Fe²⁺) heme irons.⁶ In erythrocytes, hemoglobin is continually oxidized to MHb and is actively reduced by reductase enzymes, maintaining a low concentration of methemoglobinemia. Recently, investigators in our institute found substantial (20%) benzocaine-induced methemoglobinemia in Dorset/Finn sheep after topical application of benzocaine.^{7,8} This finding, which could influence research findings, underscores the importance of characterizing the frequency and extent of methemoglobinemia induced in other laboratory animal species. Although the likelihood of serious tissue anoxia secondary to methemoglobinemia in clinically normal animals is slight,⁶ methemoglobinemia could constitute a danger for stressed or ill animals. Other commonly used laboratory animals (mice, rats, guinea pigs, rabbits) are known to vary in susceptibility to drug-induced MHb formation and clearance attributable to differences in drug metabolism, disposition, and erythrocyte susceptibility to oxidant stress.⁹ To the authors' knowledge, data are not available concerning systemic studies of common topically applied anesthetics in research animals.

Benzocaine and 20% benzocaine are commonly used and have documented potential to induce methemoglobinemia in some species, as evidenced by sev-

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This research was conducted in accordance with the principles set forth in the National Institutes of Health Publication No. 85-23, *Guide for the Care and Use of Laboratory Animals*, the 1986 Report of the AVMA Panel on Euthanasia, and the Animal Welfare Act of 1985, as amended.

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eral well-documented case reports and small clinical studies.^{1,10} The biologic significance of such observations in terms of frequency and extent of methemoglobinemia has not been evaluated in several species in any systematic manner to our knowledge.

Materials and Methods

Animal care—Adult male and female animals, identified by tattoo or ear tag, were used for this study. A minimum of 6 animals of each species were used, with the exception of the cats. Owing to documented variability of response to benzocaine in cats, 38 cats were tested. All animals were maintained in accordance with established guidelines recommended for each species.¹¹ Animals studied were adult mixed-breed dogs ($n = 11$), weighing 22 to 32 kg; adult, domestic shorthair cats ($n = 38$), weighing 2.6 to 6.3 kg; adult Long-Evans rats ($n = 11$), weighing 270 to 365 g; adult Sprague-Dawley (CrI:CD[SD]BR) rats ($n = 10$), weighing 124 to 290 g; adult ferrets ($n = 6$), weighing 2.5 to 2.8 kg; adult rhesus monkeys ($n = 10$), weighing 3.5 to 5.0 kg; adult owl monkeys ($n = 10$), weighing 850 to 974 g; adult cynomolgus monkeys ($n = 10$), weighing 3.35 to 4.8 kg; adult New Zealand White (NZW) rabbits (Hra:[NZW] specific-pathogen-free *Pasteurella* pathogen-free, $n = 18$), weighing 2 to 5 kg; adult miniature pigs ($n = 9$), weighing 24 to 59 kg; and adult mice of several stocks and strains (Hsd:ICR, C3H, C57BL/10SnJ, CrI:CD1; $n = 22$), weighing 16 to 50 g. At the beginning of the study, animals were chemically restrained by administration of a single dose of xylazine and ketamine, adjusted for species and weight. Observations in this laboratory have indicated that these drugs do not affect MHB formation. Indwelling catheters were placed aseptically in pigs, rabbits, cats, and ferrets. Catheters were maintained by use of heparinized saline flushes between blood sample collections. Blood samples from monkeys and dogs were collected via venipuncture, alternating limbs for serial sample collections. Samples were obtained from mice and rats retro-orbitally, alternating eyes for the serial sample collections. Animals were monitored during the entire sample collection period. Body temperature, mucous membrane color, capillary refill time, pulse, and respiratory rate were observed every 10 minutes until animals were able to maintain sternal recumbency, with the exception of the mice, rats, and ferrets. All mice and rats were euthanatized by cervical dislocation followed by creation of bilateral pneumothorax after the last sample collection; ferrets were euthanatized by an overdose of pentobarbital given IV.

Drug administration—Benzocaine-topical anesthetic aerosol spray^a was applied to oral mucous membranes. The bottle concentration of 14% benzocaine per 200 mg of residue allows approximately 56 mg of benzocaine to be deposited with a 2-second burst of spray applied to intact mucous membranes. This spray also contains 2% butyl aminobenzoate,

2% tetracaine hydrochloride, 0.5% benzalkonium chloride, and 0.005% cetyltrimethylammonium bromide. All animals, except mice and rats, received a 2-second spray of benzocaine-containing anesthetic after the cannula was inserted into the oral cavity, for an estimated dose of 56 mg. A 2-second spray in the rodent's oral cavity delivered too great a volume of fluid for these animals; therefore, an equivalent dose (3 drops from a syringe with a 23-gauge needle) was applied to the mucous membranes. All animals were dosed from the same benzocaine-containing spray bottle. Significant differences in delivered dose of benzocaine were not observed at the beginning or end of the study.

Sample collection and analysis of MHB—Blood samples for MHB analysis were collected (100 μ l from mice and rats; 1.0 ml from all other species) anaerobically in lithium heparin-containing syringes^b or in sodium heparinized microhematocrit capillary tubes^c for mice and rats. Samples were collected prior (time zero) to dosing with benzocaine-topical anesthetic aerosol spray^a and at 15, 30, 45, and 60 minutes after application. Samples were placed on ice until analyzed, usually within 60 minutes of collection. Methemoglobin concentration was measured by use of an oximeter^d with dedicated spectrophotometric analysis for appropriate species and appropriate controls and blanks. This instrument has the capability to simultaneously measure hemoglobin, oxyhemoglobin, carboxyhemoglobin, and volume percentage of oxygen (mmol/L), as well as MHB, with coefficient of variability < 5%.¹²

Statistical analysis—The experimental design comprised the smallest number of animals possible; however, a larger number of cats was needed because of their documented variability of response to benzocaine.¹ Descriptive statistics were used to characterize MHB response of each group of animals. The range, median, mean, and SD of responders (R) vs poor responders (PR) of each group of animals were tabulated. Responders were prospectively defined as having > 3 SD increase in MHB concentration from baseline value—usually a several-fold increase—for any subsequent post treatment sample. Animals labeled PR had a smaller, but significant, increment from reference baseline MHB values. Time/MHB blood concentration graphs were plotted for each animal. An area under the MHB response curve from 0 to 60 minutes (AUC_{0-60}) was calculated as the product of the mean percentage of MHB and sample collection time interval (t in minutes), using the trapezoidal rule: $AUC_{0-60} = [(MHB_1 + MHB_2)/2] \times (t_2 - t_1)$.

Results

Response to topically applied benzocaine-containing anesthetic was almost universal across groups. The magnitude of response, inter- and intragroups, was highly variable (Fig 1). Mice data were pooled

^a Cetacaine, Cetylite Industries Inc, Pennsauken, NJ.

^b Marquest Medical Products Inc, Englewood, Colo.

^c Allied Corp, Fisher Scientific, Pittsburgh, Pa.

^d Radiometer, Radiometer America Inc, Westlake, Ohio.

because of similarity of response among all strains. Analysis of mean response between mice species/stock was not significant. Typical MHB response curves after anesthetic application were constructed for 6 rabbits: clear populations of R and PR were evident (Fig 2). Similar findings were seen in cats and rats. Variability in mean time to peak MHB also was observed among groups (Fig 3). Time (minutes) to peak (mean \pm 1 SD) MHB response varied from 18.2 ± 6.4 minutes for mice to 45.0 ± 16.8 minutes for pigs; usually, but not uniformly, response decreased toward reference values by 60 minutes. Animals were dosed similarly; PR remained PR on 4 observations after drug application; therefore, it is unlikely that a brief response was missed. With the exception of dogs, all groups of animals had R with > 3 SD above baseline individual and group MHB means and values for PR. Testing of dogs was repeated several months later to confirm low response. Dogs responded to parenter-

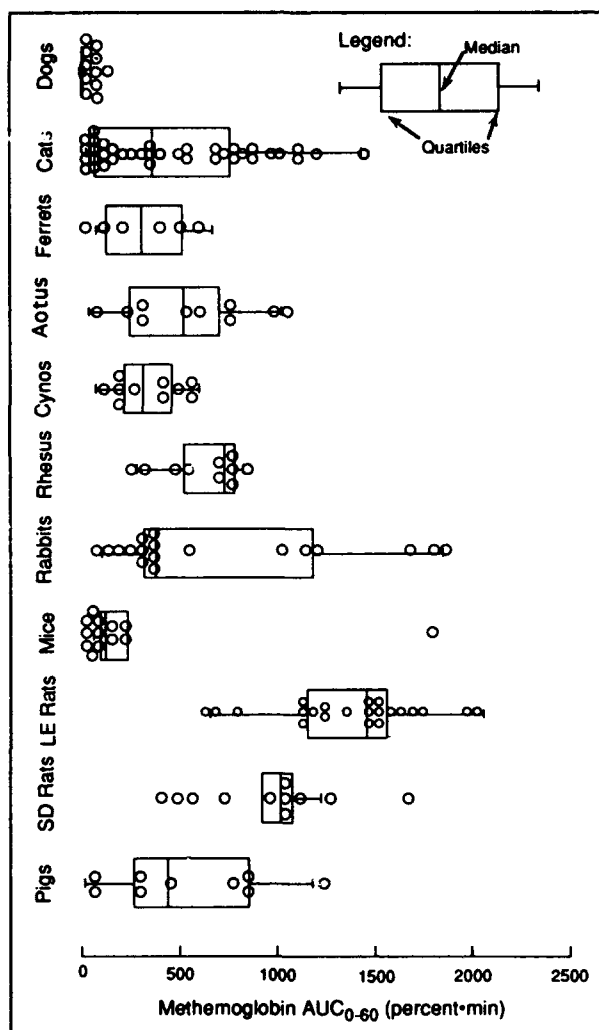


Figure 1—Box plots with individual data points for 11 species administered topically applied benzocaine-containing anesthetic. Individual data points represent total area under the percentage of methemoglobin (MHB) time curve from 0 to 60 minutes for each animal (AUC_{0-60}). For each species group, the median and midquartile (25%) are indicated by the group boxes. Although highly variable, MHB response can be observed within every species group. SD = Sprague-Dawley; LE = Long-Evans rats.

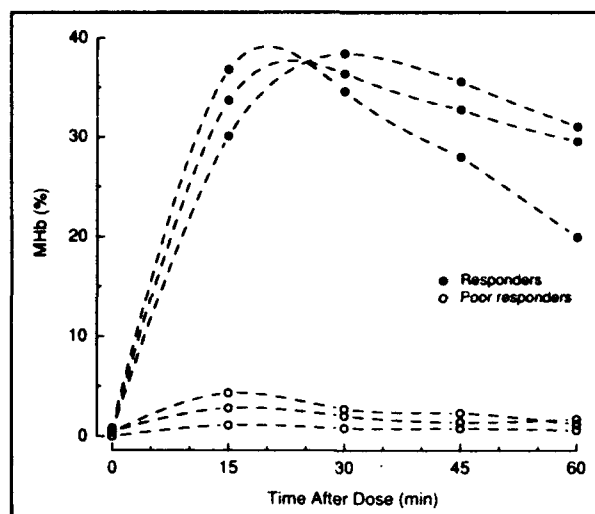


Figure 2—Percentage of MHB concentration time plots for 3 rabbits (●) with a marked positive response to topically applied benzocaine (spray) and 3 rabbits (○) with limited response to the same dose. Increase to peak MHB at 15 minutes or 30 minutes and subsequent decrease to 60 minutes can be seen.

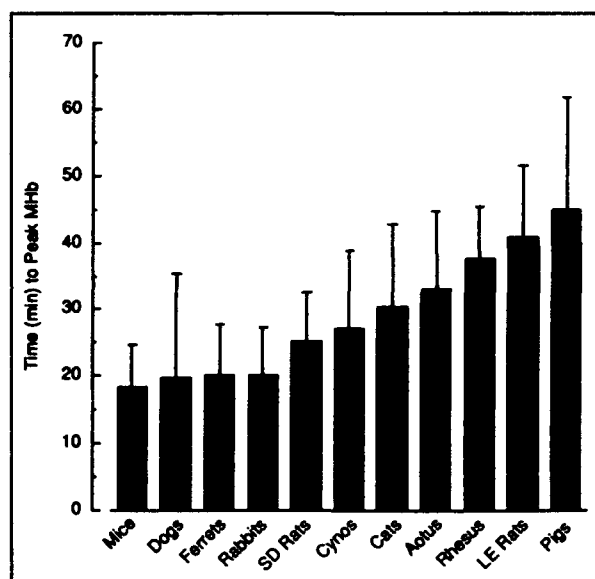


Figure 3—Time to peak MHB concentration in species groups. Time to peak (mean \pm 1 SD) is indicated for each of the species groups and illustrates wide range in rate of response between groups. See Figure 1 for key.

ally (IV) administered benzocaine^c (data not shown). Although the dogs' response was less than that of other animals (< 3 SD above baseline), they had a consistent, albeit small, response to topically applied benzocaine. We confirmed that dogs had a moderate, but significant, response to the topically administered spray by results of a separate, repeat study with appropriate controls. We did not believe it necessary to repeat the test on all animals.

Discussion

Magnitude of response depends on potency of the oxidant and its duration of action. Methemoglo-

^c Hurricane, Beutlich Inc, Niles, Ill.

bin-forming compounds are generally aromatic amines: aminophenols or N-hydroxyarylamines (Fig 4). The latter compounds are the most potent MHb formers. Although the topically applied benzocaine contains several chemicals, it most likely causes MHb formation.⁷ Benzocaine, an ethyl ester of *p*-aminobenzoic acid, is a local anesthetic that probably is metabolized to *p*-aminobenzoic acid and ethanol by plasma and liver esterases.⁷ *p*-Aminobenzoic acid induces Heinz body formation in cats, but has not been shown to induce MHb formation. However, many benzocaine-based products have been strongly associated with methemoglobinemia.^{2,4,13} The close association of MHb formation after topical application of benzocaine-containing products, as well as lack of other potential oxidants, indicates a possible cause-effect relation of benzocaine and MHb. *p*-Aminobenzoic acid undergoes N-hydroxylation to N-hydroxy *p*-aminobenzoic acid.^{9,11,15} This N-hydroxy derivative may then oxidize hemoglobin to MHb. N-Hydroxylation is the most likely common and critical step that produces the MHb-forming metabolites of benzocaine. Guertler et al⁷ support this speculation; sheep that responded to benzocaine also responded to *p*-aminopropiophenone, where a known active metabolite is *p*-hydroxyaminopropiophenone.¹⁵ Genetic variation in the N-hydroxylation pathways would explain inter- and intraspecies differences in MHb response to benzocaine. Visual analysis of MHb curves for individual animals indicated that for several species, MHb elim-

ination was similar to rates described in the aforementioned more-detailed studies.

Dogs, as a group, had the smallest, but still significant ($P < 0.05$), increment from reference baseline MHb values. It is not possible, on the basis of data from these studies, to confidently state whether the smaller response in dogs is attributable to differences in absorption, MHb formation, or more rapid clearance. Results of our study regarding comparatively lower response in dogs is of interest, however, because this species is stated to have MHb response most similar to that in human beings.^{8,16}

In conclusion, substantial increase in MHb concentration was seen in several animal species after a standard topically applied dose of a benzocaine-containing topical anesthetic. Poor responders of certain species and individual animals probably represent differences in drug metabolism, as well as absorption and elimination. Use of topically applied anesthetics can cause unrecognized, but physiologically significant, changes. The extent of drug-induced MHb changes in many of the species of this study could have at least 2 types of effects on in vivo models. Methemoglobin, even in low concentration (10%), results in a direct decrement in O₂-carrying capacity of erythrocytes, which can affect performance studies.⁸ Other, more subtle changes in cardiorespiratory or endocrine/metabolic responses are possible with the higher MHb values ($\geq 20\%$) seen in this study. Secondly, MHb formation is an expression of oxidant stress to erythrocytes. Related changes of drug-induced hemolysis are seen in sensitive species (sheep).

With these data in mind, investigators can consider an alternative topically applied anesthetic (lidocaine). This drug, however, has its own well-described toxicity to neurologic or cardiac systems in higher doses.

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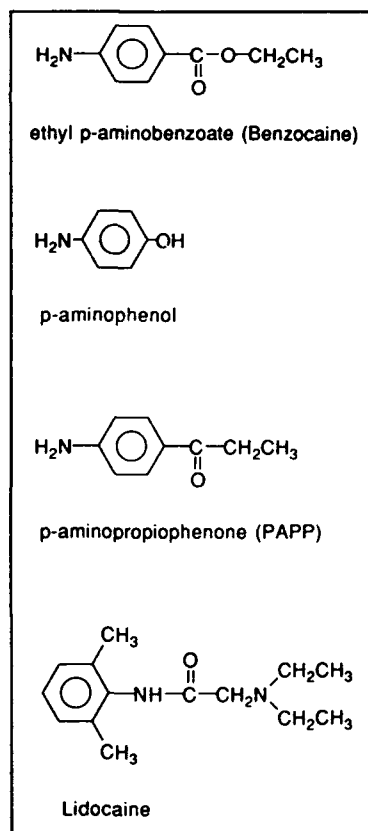


Figure 4—Structures of 3 aromatic amine drugs associated with MHb formation and a fourth compound, lidocaine, not associated with MHb formation in usually administered doses.

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